Novel Strategies for the Identification of Soil Bacteria Producing Antimicrobial Compounds
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Introduction
Clostridium difficile is a pathogen that causes PMC (pseudomembranous colitis), a life threatening infection of the colon. PMC is characterized by severe inflammation of colonic tissues. PMC can progress suddenly to fulminating colitis and Toxic Megacolon (TM) which both often result in death. Infections of C. difficile occur when broad spectrum antibiotics wipe out the protective normal flora on colonic tissues. C. difficile forms highly resistant endospores that later germinate uncontrolled when conditions become favorable. C. difficile produces 2 exotoxins, TsoA (enterotoxin), and TsoB (cytotoxin). The latter activates the intestinal mucosa. TsoB (cytotoxin) cannot penetrate the intestinal mucosa independent of TsoA, but it is 10,000 times more toxic to cells. The CDC reports that the northeastern region of the United States (includes CT) has the highest rate of C. difficile infection per patient in the nation. Furthermore, the NAP-1 strain of C. difficile (previously only endemic to Europe and Canada) was recently found in New York City patients. NAP-1 produces 16X more enterotoxin, and 23X more cytotoxin than a common strain of C. difficile.

In this investigation, Clostridium sphenogonous is used as a non-pathogenic surrogate for susceptibility analysis of C. difficile. In this way, tests can be performed safely in the university laboratory. Members of the Clostridium genus are Gram-positive, spore forming, motile, and anaerobic. The purpose of this research is to discover new antibiotics from soil cultures that may inhibit the growth of C. difficile. An antibiotic disk test was used to verify the suitability of C. sphenogonous as a surrogate for C. difficile. A slide stripe test was used to determine the susceptibility of Clostridia to several unknown inhibitory compounds.

Methods

Antibiotic Disk Test
Commercially produced disks containing clindamycin, penicillin, doxycycline, vancomycin, streptomycin (and a blank disk) were placed on NA (Nutrient Agar) and BHI (Brain Heart Infusion Agar) plates immediately after inoculation of C. sphenogonous by the spread plate technique. Plates were incubated anaerobically at 37°C for 4 days.

Results

Antibiotic Disk Test (BHI Plates Shown)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>C. sphenogonous</th>
<th>C. pathogenes</th>
<th>C. kefyr</th>
<th>C. cerevisiae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank Disk</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>Penicillin</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
</tr>
</tbody>
</table>

Question and Future Directions

Antibiotic disk testing indicates that this strain of C. sphenogonous mimic the susceptibility of a common C. difficile strain.1 The inhibitory property of the TAS-10 exotoxin (on Clostridia) merits further investigation. In future testing, we aim to determine the molecular size of this unknown compound, molecular stability, and the type of biomolecule. We could then derive methods to purify the molecule and eventually identify it. The purified chemical could then be better compared in killing to established antibiotics such as vancomycin, metronidazole, or perhaps even new synthetic drugs such as Inebiol (an exoxidazin).

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